Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans


1Department of Endocrinology and Diabetes, University Hospital of Aarhus, Aarhus Kommunehospital, and Institutes of Experimental Clinical Research and Clinical Pharmacology, University of Aarhus, DK-8000 Aarhus, Denmark.

Received 7 December 2001; accepted in final form 4 March 2002

Djurhuus, C. B., C. H. Gravholt, S. Nielsen, A. Mengel, J. S. Christiansen, O. E. Schmitz, and N. Møller. Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. Am J Physiol Endocrinol Metab 283: E172–E177, 2002; 10.1152/ajpendo.00544.2001.—Cortisol’s effects on lipid metabolism are controversial and may involve stimulation of both lipolysis and lipogenesis. This study was undertaken to define the role of physiological hypercortisolemia on systemic and regional lipolysis in humans. We investigated seven healthy young male volunteers after an overnight fast on two occasions by means of microdialysis and palmitate turnover in a placebo-controlled manner with a pancreatic pituitary clamp involving inhibition with somatostatin and substitution of growth hormone, glucagon, and insulin at basal levels. Hydrocortisone infusion increased circulating concentrations of cortisol (888 ± 12 vs. 245 ± 7 nmol/l). Interstitial glycerol concentrations rose in parallel in abdominal (327 ± 35 vs. 156 ± 30 nmol/l; P = 0.05) and femoral (178 ± 28 vs. 91 ± 22 nmol/l; P = 0.02) adipose tissue. Systemic [3H]palmitate turnover increased (165 ± 17 vs. 92 ± 24 μmol/min; P = 0.01). Levels of insulin, glucagon, and growth hormone were comparable. In conclusion, the present study unmistakably shows that cortisol in physiological concentrations is a potent stimulus of lipolysis and that this effect prevails equally in both femoral and abdominal adipose tissue.

The potential role of cortisol in the regulation of lipid metabolism is a matter of debate. Whereas it is well established that glucocorticoids induce insulin resistance (2, 6) and promote proteolysis (13), studies in the field of intermediary lipid metabolism have yielded more conflicting results.

Recent in vitro studies have suggested that cortisol inhibits basal and catecholamine-stimulated lipolysis in cultured human adipocytes from abdominal tissue (23, 24), although these studies were long-term incubations carried out in the course of days rather than hours. On the other hand, in vivo experiments using [3H]palmitate infusion reported that high physiological levels of cortisol led to 60% increases in free fatty acid (FFA) concentrations and palmitate flux, an indicator of effective adipose tissue lipolysis, in humans (7). Partly in line with these findings, Samra et al. (30) observed increased circulating FFA concentrations and an increased systemic appearance rate for glycerol during hypercortisolemia but, concurrently, a decrease in FFA efflux from abdominal tissue. Taken together, these observations may be compatible with the notion that, although stimulating overall lipolysis at the whole body level, glucocorticoids may specifically inhibit abdominal lipolysis. This hypothesis gains further support from the clinical observation that patients with chronic hypercortisolemia secondary to Cushing’s syndrome are characterized by distinct abdominal obesity (19, 26, 35).

Microdialysis allows continuous monitoring of changes of concentrations of a variety of low molecular weight compounds from interstitial tissue to the microdialysate and, hence, estimation of regional concentrations in various tissues. The technique has been applied in a large number of studies in humans since it was first introduced (20).

The present study was undertaken to examine the effect of an acute physiological elevation of circulating cortisol concentrations on systemic and regional adipose tissue. In particular, we addressed the question of whether cortisol preferentially stimulates lower body adipose tissue lipolysis as opposed to abdominal lipolysis. To pursue this, we examined seven subjects with or without concomitant hydrocortisone infusion during infusion of somatostatin to prevent ensuing differences in hormone secretion, in particular alterations in insulinemia.

MATERIALS AND METHODS

Before the study, we performed a power analysis by use of an estimated 60% increase in serum FFA, found by Divertie et al. (7), and a power of 80%, α = 0.05. On the basis of this analysis, we included seven healthy young males [age 27 ± 2.5 SE (range 25–31); body mass index 24.0 ± 1.6 (range 21.3–27.9); height 177.8 ± 7.3 cm (range 170–182 cm); weight 73.8 ± 10.3 kg (range 66–86 kg)]. The oral glucose tolerance test was carried out after a 10-h overnight fast. Blood samples were obtained every 20 min for measurement of plasma glucose and insulin. After the oral glucose tolerance test, an arterial catheter was inserted in a hand vein for measurement of cortisol and catecholamines. The fasting serum cortisol was decreased, with the mean value being 200 ± 36 nmol/l (range 122–295). The baseline serum FFA was 0.09 ± 0.02 μmol/l (range 0.05–0.14). In the first part of the study, subjects received saline (500 ml) over a 4-h period. In the second part of the study, subjects received saline (500 ml) over a 4-h period followed by hydrocortisone (200 mg i.v.) on the same day. The cortisol was given to all subjects at a rate of 0.5 mg/h for 4 h. Plasma cortisol concentrations were measured every 30 min. Plasma levels of cortisol increased in parallel with the infusion rate in all subjects. The mean plasma cortisol level was 316 ± 57 nmol/l (range 260–394) after 30 min, 370 ± 56 nmol/l (range 312–454) after 60 min, 396 ± 58 nmol/l (range 346–455) after 90 min, and 398 ± 57 nmol/l (range 346–455) after 120 min. An analysis of variance showed a significant increase (P < 0.001) in plasma cortisol concentrations. After the glucose load, plasma FFA increased in parallel, with a mean value of 0.71 ± 0.12 μmol/l (range 0.55–0.86). The FFA increased (P < 0.001) by 4.8 ± 0.7 μmol/l (range 3.2–7.7) after the glucose load. The interstitial glycerol concentration increased in parallel in abdominal and femoral tissue. The mean increase was 3.2 ± 0.7 μmol/l (range 1.6–4.9) in abdominal tissue and 1.3 ± 0.3 μmol/l (range 0.7–2.3) in femoral tissue. The interstitial glycerol concentration increased (P < 0.001) by 2.8 ± 0.6 μmol/l (range 1.6–4.5) in abdominal tissue and 0.9 ± 0.2 μmol/l (range 0.5–1.6) in femoral tissue.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
of [2H3]palmitate as internal standard (16). Systemic palmitate flux was calculated using the 9,10-3H]palmitate infusion rate divided by the steady-state palmitate SA.

Indirect calorimetry. Indirect calorimetry was performed with a ventilated hood (Deltatrac Metabolic Monitor; Datex, Helsinki, Finland) at 40 l/min. Energy expenditure, respiratory quotient, and 24-h excretion of urea were measured from the excretion rate of urea in urine collected during the entire study period, and glucose, protein, and lipid oxidation were calculated (8). Calibration of the system was performed by combustion of a known amount of 99.6% ethanol at regular intervals. Baseline calorimetry was performed at baseline after 30 min of rest and was carried out for 30 min. Stimulated calorimetry was initiated 30 min before the end of the experiment.

Adipose tissue blood flow. To ensure stable tissue perfusion conditions, subcutaneous adipose tissue perfusion was measured with the 133Xe washout technique (17). Approximately 3 Mq of 133Xe were injected into the subcutaneous adipose tissue of the abdomen ~5 cm sinistrolateral to the umbilicus. Disappearance of 133Xe was continuously measured starting from 30 min after injection by use of a NaI detector (EG&G Ortec, Workingham, Berkshire, UK), as previously described (10). The tissue-to-blood partition coefficient was corrected for skinfold caliper as described by Bülow et al. (5).

Assays. PG was analyzed in duplicate with the glucose oxidase method (Beckman Coulter, Palo Alto, CA). Measurements were performed immediately to determine isotonic glucose infusion rate.

Serum GH was analyzed with a double monoclonal immunofluorometric assay (Delfia, Wallac Oy, Turku, Finland). Serum C-peptide and insulin were measured with an immunoassay (Dako, Glostrup, Denmark). Plasma glucagon was measured by an in-house radioimmunoassay (22). Serum cortisol was measured with a solid-phase time-resolved fluorimunoassay (Delfia, Wallac Oy).

Serum FFA was determined by a colorimetric method with a commercial kit (Wako Pure Chemical Industries, Neuss, Germany).

Blood levels of alanine, glycerol, 3-OHB, and lactate were assayed with an automated fluorometric method (18).

Glyceral levels in the microdialysis dialysate were measured in duplicate by an automated spectrophotometric kinetic enzymatic analyzer (CMA 600, CMA, Solna, Sweden).

Statistical analysis. Results are expressed as time-averaged values during baseline (t = 150–0) and the stimulated period (t = 240–360 min). Statistical difference is based on comparison between the area under the curve (AUC) during baseline (t = 150–0) and that during the stimulated period (t = 240–360 min) unless otherwise specified.

AUC was calculated using ICPilot version 1.0 (CMA Stockholm) with the trapezoid method. Statistical analysis was performed using SPSS for Windows version 10.0 (SPSS, Chicago, IL).

The Kolmogorov-Smirnov test of normal distribution of the data was performed, and depending on the outcome, a parametric (Student’s t-test for paired samples) or Wilcoxon signed ranks test (for related samples) was used. P values <0.05 were considered significant.

RESULTS

Circulating hormones. During hydrocortisone sodium succinate infusion, serum cortisol rose to 888 ± 12 vs.
245 ± 7 nmol/l in the placebo situation (Fig. 1). Serum levels of insulin remained stable, ~30 pmol/l, in both experiments, as did plasma glucagon in the range of 40 ng/l. During infusion of somatostatin, serum C-peptide concentrations declined in both situations, but less so during hydrocortisone infusion (P = 0.03; Table 1). Comparable replacement levels of insulin and glucagon were recorded (Table 1): insulin (baseline (P = 0.60); stimulation (P = 0.76]) and glucagon (baseline (P = 0.50); stimulation (P = 0.74]).

Serum levels of GH were subtly increased before (P = 0.02) and during hydrocortisone administration (P = 0.02; Table 1).

**Circulating metabolites.** PG levels were similar in both circumstances, at baseline and after stimulation (P > 0.3; Table 2). Serum FFA increased from 0.320 ± 0.01 to 0.612 ± 0.01 mmol/l (AUC0–360 difference P = 0.015).

**Interstitial glycerol concentrations.** In the abdominal subcutaneous adipose tissue, the interstitial glycerol increased to 327 ± 35 vs. 156 ± 30 μmol/l, corresponding to an average increase of 110% (AUC0–240–360 difference P = 0.05; Table 3; Fig. 2). Femoral interstitial glycerol concentration increased to 178 ± 28 vs. 91 ± 22 μmol/l, equal to an increase of 96% (AUC0–240–360 difference P = 0.02). No significant difference was found between the abdominal and femoral increases (P = 0.23).

**Palmitate metabolism.** Baseline palmitate fluxes (Fig. 3) were comparable in both situations (P = 0.24), whereas palmitate fluxes increased during hydrocortisone infusion (P = 0.01).

**Indirect calorimetry.** Lipid oxidation determined by indirect calorimetry showed no difference between baseline situations [518 ± 17 vs. 518 ± 33 kcal/24 h (P = 1.00; Table 4)], whereas a profound increase in lipid oxidation was found during hydrocortisone infusion [850 ± 31 vs. 564 ± 40 kcal/24 h (P = 0.02)].

---

### Table 1. Hormones

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Cortisol</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, pmol/l</td>
<td>Baseline 29.96 ± 1.14</td>
<td>31.06 ± 1.74</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>Stimulated 33.93 ± 1.50</td>
<td>31.93 ± 1.49</td>
<td>0.76</td>
</tr>
<tr>
<td>C-peptide, pmol/l</td>
<td>Baseline 205.60 ± 19.59</td>
<td>207.19 ± 20.71</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Stimulated 40.74 ± 7.00</td>
<td>86.36 ± 11.49</td>
<td>0.03</td>
</tr>
<tr>
<td>GH, μg/l</td>
<td>Baseline 0.36 ± 0.04</td>
<td>0.62 ± 0.11</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Stimulated 0.54 ± 0.03</td>
<td>0.83 ± 0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>Baseline 44.83 ± 7.44</td>
<td>40.54 ± 5.54</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Stimulated 29.19 ± 6.68</td>
<td>28.41 ± 6.67</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Data are time-averaged values during baseline (t = −150–0 min) and stimulation (t = 240–360 min); paired analysis of area under the curve (AUC) during baseline and stimulation. GH, growth hormone.

### Table 2. Circulating metabolites

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Cortisol</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine, µmol/l</td>
<td>Baseline 276 ± 14</td>
<td>246 ± 10</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Stimulated 320 ± 15</td>
<td>270 ± 9</td>
<td>0.12</td>
</tr>
<tr>
<td>3-OHB, µmol/l</td>
<td>Baseline 40 ± 8</td>
<td>43 ± 8</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Stimulated 44 ± 11</td>
<td>94 ± 10</td>
<td>0.29</td>
</tr>
<tr>
<td>Lactate, µmol/l</td>
<td>Baseline 619 ± 38</td>
<td>545 ± 22</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Stimulated 680 ± 30</td>
<td>582 ± 20</td>
<td>0.08</td>
</tr>
<tr>
<td>Glycerol, µmol/l</td>
<td>Baseline 37 ± 3</td>
<td>37 ± 3</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Stimulated 34 ± 3</td>
<td>51 ± 3</td>
<td>0.03</td>
</tr>
<tr>
<td>[9,10-3H]palmitate, µmol/min</td>
<td>Baseline 81 ± 17</td>
<td>105 ± 33</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Stimulated 92 ± 24</td>
<td>165 ± 17</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Data are time-averaged values during baseline (t = −150–0 min) and stimulation (t = 240–360 min); paired analysis of AUC during baseline and stimulation. 3-OHB, 3-hydroxybutyrate.
Glucose infusion rate and blood flow. Glucose infusion, measured as glucose infusion rate (mg/h), was comparable: 21.45 ± 5.70 mg (saline) and 20.43 ± 6.03 mg (hydrocortisone; P = 0.93). Adipose tissue blood flow (ATBF) was equal during baseline [3.53 ± 0.52 ml/100 mg·min⁻¹ (saline)] vs. 3.30 ± 0.89 ml/100 mg·min⁻¹ (hydrocortisone); P = 0.94] and during stimulation [4.29 ± 0.99 ml/100 mg·min⁻¹ (saline)] vs. 3.14 ± 0.53 ml/100 mg·min⁻¹ (hydrocortisone); P = 0.45].

DISCUSSION

The aim of this study was to determine the effects of acute hydrocortisone sodium succinate and subsequent elevations of circulating cortisol on total and local FFA and glycerol mobilization in the postabsorptive state. In line with previous studies (6, 7), we found that cortisol increased serum FFA, serum glycerol, and whole body palmitate turnover, indicating increased systemic lipolysis. In addition, we observed that cortisol augmented lipid oxidation (assessed by indirect calorimetry) and led to proportionate increments in interstitial glycerol concentrations in abdominal and femoral adipose tissue, findings that to our knowledge are novel. On the whole, our results suggest that cortisol stimulates upper body and lower body subcutaneous adipose tissue lipolysis evenly and does not support the notion that glucocorticoids per se cause abdominal obesity by preferentially inhibiting regional upper body lipolysis. It should be emphasized that, with the present design, we accomplished cortisol concentrations in the high physiological range (32) in the presence of comparable levels of insulin and glucose.

In our study, we recorded insulin concentrations of ~30 pmol/l, close to normal fasting levels of between 20 and 30 pmol/l, whereas the glucagon values of ~40 ng/ml were decreased compared with normal fasting levels.
levels of between 60 and 70 ng/ml (11). Thus a relative lack of glucagon may explain the need to administer small amounts of exogenous glucose temporarily at a rate of ~20 mg/h to maintain euglycemia; it is unlikely that the glucagon deficit has affected lipid metabolism, because glucagon has no detectable effect on lipolysis (10).

Circulating GH levels were minutely increased in our study. Although it is likely that glucocorticosteroids may interfere with GH secretion, the fact that GH levels were increased before cortisol administration suggests that the difference has been accidental rather than cortisol induced. GH has lipolytic effects (11), but it is unlikely that tiny GH elevations of 0.2–0.3 μg/l in the circulation have had any major impact on lipolysis.

Microdialysis measures flux-generating concentrations of a variety of compounds across a diminutive dialysis membrane and permits assessment of changes in interstitial concentrations of these compounds in various tissues (4, 14, 20, 28, 34). True (or “quasi-true”) equilibrium across the membrane is only accomplished with very low flow rates, as exploited in the present study (28). Under these circumstances, any increase in glycerol concentrations in the perfusate may be seen as a reflection of increased regional lipolysis, provided local blood flow and glycerol clearance are not altered.

In the present study, we observed comparable increments in interstitial glycerol concentrations in femoral and abdominal adipose tissue during cortisol exposure. This observation is at variance with previous suggestions that cortisol may inhibit lipolysis in cultured abdominal adipocytes in vitro (23, 24) and diminish FFA efflux from abdominal adipose tissue in vivo (30).

In this context, it should be considered that any change in regional blood flow could alter the flux-generating concentration gradients across the dialysis catheter. We did not, however, observe any change in ATBF during cortisol exposure. This finding is in line with the study of Samra et al. (30). In addition, we utilized a low perfusion rate in our study, meaning that the results were obtained at quasi-equilibrium and that any change in relative recovery has been of minor importance.

Furthermore, a concomitant increase in the rate of lipolysis and of reesterification of FFA to triacylglycerol in abdominal adipocytes could lead to an increased glycerol release and a decreased or unchanged FFA release. Still, the study of Samra et al. (30) failed to observe any indications that the ratio of FFA to glycerol in efferent subcutaneous blood changed during cortisol exposure.

The prevailing concentrations of glucose and, hence, insulin in the circulation are evidently crucial; it is well described that lipolysis is exquisitely sensitive to minute changes in insulinemia (15, 29). With the present design, we accomplished virtually identical levels of insulin and glucose by infusing somatostatin (to inhibit endogenous insulin secretion) and glucose to maintain euglycemia. In some previous studies (30), a distinct possibility was raised that minute elevations in circulating glucose and insulin concentrations might have inhibited lipolysis in some regions.

It should be highlighted that our results, including the observation of stimulated abdominal adipose tissue lipolysis, represent the direct and acute effects of cortisol. With prolonged elevation of circulating glucocorticoid levels, insulin resistance and subsequent hyperinsulinemia evolve, and it is indeed conceivable that the combination of hypercortisolemia and hyperinsulinemia may preferentially inhibit upper body lipolysis, as suggested in the studies by Samra et al. (30).

Furthermore, it should be noted that the arteriovenous difference study by Samra et al. (30) employed a hydrocortisone infusion of 11 h, and our study had a duration of merely 6 h. Additionally, supraphysiologically levels of cortisol were obtained (1,500 nmol/l) in the former study, whereas we accomplished cortisol levels comparable to severe stress (~800 nmol/l).

As discussed elsewhere, microdialysis and the arteriovenous technique developed by Frayn et al. (9) and exploited in the study of Samra et al. (30) are two distinct methods with complementary applications in the study of adipose tissue metabolism (4, 33). For instance, the arteriovenous technique includes substantial components of skin metabolism and intravascular lipoprotein lipase (LPL) activity; in regard to quantitative measurements of flux rates, both methods are hampered by inability to measure blood flow rates in the relevant tissue compartments (4, 33). Theoretically, it is thus possible that some of the findings in studies employing arteriovenous techniques could relate to changes in either the activity or the effectiveness of LPL.

Alternative mechanisms whereby cortisol could promote deposition of upper body fat include specific stimulation of lipogenesis. Finally, it is a vexing question how cortisol affects visceral adipose tissue. This lipid pool is not readily accessible, and it is possible that cortisol specifically may inhibit lipolysis in the region.

The intracellular mechanisms behind the metabolic effects of glucocorticoids are uncertain. Cortisol acts at the intracellular glucocorticoid receptors that are present in significant numbers in adipocytes (25, 27). Studies in human and rat adipocytes have suggested that glucocorticoids may stimulate both LPL and hormone-sensitive lipase (HSL) activity (24, 31). On the other hand, the study of Samra et al. (30) indicated inhibition of HSL in adipose tissue. The apparent discrepancy could relate to minute elevations of circulating insulin concentrations in the latter study, the rate of lipolysis being extremely sensitive to insulin.

In conclusion, our results clearly suggest that cortisol in physiological concentrations stimulates whole body lipolysis and that this effect is caused by proportionate stimulation of femoral and abdominal subcutaneous adipose tissue. To what extent these events may be modulated by ensuing hyperinsulinemia remains uncertain.

Roche Diagnostics is kindly thanked for donation of the microdialysis catheters.
REFERENCES


